JC17 Rec'd PCT/PTO 29 JUN 2001
CHAPTER II 09/869597

Preliminary Classification:
Proposed Class:
Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P., § 601, 7th ed.

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US) (ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/04438	24 December 1999	29 December 1998

TITLE OF INVENTION
A METHOD OF TESTING A CELL SAMPLE

APPLICANT(S)
Ian Basil SHINE, Thomas Adam SHINE

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

CERTIFICATION UNDER 37 C.F.R. §§ 1.8(a) and 1.10*
*(When using Express Mail, the Express Mail label number is **mandatory**; Express Mail certification is optional.)*

I hereby certify that, on the date shown below, this correspondence is being:

MAILING

☒ deposited with the United States Postal Service in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

37 C.F.R. § 1.8(a)

☐ with sufficient postage as first class mail.

37 C.F.R. § 1.10 *

☒ as "Express Mail Post Office to Addressee"
Mailing Label No. EL627430875US **(mandatory)**

TRANSMISSION

☐ facsimile transmitted to the Patent and Trademark Office, (703) _____-_____.

Debra Conrad
Signature

Date: 29 June 2001

Debra G. Conrad
(type or print name of person certifying)

** Only the date of filing (§ 1.6) will be the date used in a patent term adjustment calculation, although the date on any certificate of mailing or transmission under § 1.8 continues to be taken into account in determining timeliness. See § 1.703(f). Consider "Express Mail Post Office to Addressee" (§ 1.10) or facsimile transmission (§ 1.6(d)) for the reply to be accorded the earliest possible filing date for patent term adjustment calculations.*

NOTE: To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

***WARNING:** Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:

a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
☐*	TOTAL CLAIMS 16	16 -20=	0	× $18.00=	$ 0
	INDEPENDENT CLAIMS 1	1 -3=	0	× $80.00=	0
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	
BASIC FEE**	☐ U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an international preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: ☐ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... $100.00 ☐ and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... $690.00 ☒ U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: ☐ has been paid (37 C.F.R. § 1.492(a)(2)) ..... $710.00 ☐ has not been paid (37 C.F.R. § 1.492(a)(3)) ..... $1000.00 ☒ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) $860.00				860.00
				Total of above Calculations	= 860.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Assertion must be made. (note 37 C.F.R. § 1.27)				- 430.00
				Subtotal	430.00
				Total National Fee	$ 430.00
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL				Total Fees enclosed	$ 430.00

*See attached Preliminary Amendment Reducing the Number of Claims.

☒ Attached is a ☒ check ☐ money order in the amount of $ 430.00

☐ Authorization is hereby made to charge the amount of $ _____

☒ to Deposit Account No. 16-1350

☐ to Credit card as shown on the attached credit card information authorization form PTO-2038.

WARNING: *Credit card information should* ***not*** *be included on this form as it may become public.*

☒ Charge any additional fees required by this paper or credit any overpayment in the manner authorized above.

A duplicate of this paper is attached.

"WARNING: *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

☒ **Assertion of Small Entity Status**

☒ **Applicant hereby asserts status as a small entity under 37 C.F.R. § 1.27.**

NOTE: 37 C.F.R. § 1.27(c) deals with the assertion of small entity status, whether by a written specific declaration thereof or by payment as a small entity of the basic filing fee or the fee for the entry into the national phase as states:

"(c) Assertion of small entity status. Any party (person, small business concern or nonprofit organization) should make a determination, pursuant to paragraph (f) of this section, of entitlement to be accorded small entity status based on the definitions set forth in paragraph (a) of this section, and must, in order to establish small entity status for the purpose of paying small entity fees, actually make an assertion of entitlement to small entity status, in the manner set forth in paragraphs (c)(1) or (c)(3) of this section, in the application or patent in which such small entity fees are to be paid.

(1) Assertion by writing. Small entity status may be established by a written assertion of entitlement to small entity status. A written assertion must:

(i) Be clearly identifiable;

(ii) Be signed (see paragraph (c)(2) of this section); and

(iii) Convey the concept of entitlement to small entity status, such as by stating that applicant is a small entity, or that small entity status is entitled to be asserted for the application or patent. While no specific words or wording are required to assert small entity status, the intent to assert small entity status must be clearly indicated in order to comply with the assertion requirement.

(2) Parties who can sign and file the written assertion. The written assertion can be signed by:

(i) One of the parties identified in §§ 1.33(b) (e.g., an attorney or agent registered with the Office), §§ 3.73(b) of this chapter notwithstanding, who can also file the written assertion;

(ii) At least one of the individuals identified as an inventor (even though a §§ 1.63 executed oath or declaration has not been submitted), notwithstanding §§ 1.33(b)(4), who can also file the written assertion pursuant to the exception under §§ 1.33(b) of this part; or

(iii) An assignee of an undivided part interest, notwithstanding §§ 1.33(b)(3) and 3.73(b) of this chapter, but the partial assignee cannot file the assertion without resort to a party identified under §§ 1.33(b) of this part.

(3) Assertion by payment of the small entity basic filing or basic national fee. The payment, by any party, of the exact amount of one of the small entity basic filing fees set forth in §§ 1.16(a), (f), (g), (h), or (k), or one of the small entity basic national fees set forth in §§ 1.492(a)(1), (a)(2), (a)(3), (a)(4), or (a)(5), will be treated as a written assertion of entitlement to small entity status even if the type of basic filing or basic national fee is inadvertently selected in error.

(i) If the Office accords small entity status based on payment of a small entity basic filing or basic national fee under paragraph (c)(3) of this section that is not applicable to that application, any balance of the small entity fee that is applicable to that application will be due along with the appropriate surcharge set forth in §§ 1.16(e), or §§ 1.16(l).

(ii) The payment of any small entity fee other than those set forth in paragraph (c)(3) of this section (whether in the exact fee amount or not) will not be treated as a written assertion of entitlement to small entity status and will not be sufficient to establish small entity status in an application or a patent."

3. ☒ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. ☐ is transmitted herewith.

b. ☐ is not required, as the application was filed with the United States Receiving Office.

c. ☒ has been transmitted

i. ☒ by the International Bureau.

Date of mailing of the application (from form PCT/1B/308): 7/6/00

ii. ☐ by applicant on _____ *(Date)*

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

a. ☐ is transmitted herewith.

b. ☒ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on _____. *(Date)*

d. ☐ will follow.

5. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. ☐ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau.

Date of mailing of the amendment (from form PCT/1B/308):

_____.

ii. ☐ by applicant on _____. *(Date)*

c. ☒ have not been transmitted as

i. ☒ applicant chose not to make amendments under PCT Article 19. Date of mailing of Search Report (from form PCT/ISA/210.):
7/4/00

ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☒ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the amendments were made in the English language.

c. ☒ has not been transmitted for reasons indicated at point 5(c) above.

7. ☒ A copy of the international examination report (PCT/IPEA/409)

☒ is transmitted herewith.

☐ is not required as the application was filed with the United States Receiving Office.

8. ☐ Annex(es) to the international preliminary examination report

a. ☐ is/are transmitted herewith.

b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☐ A translation of the annexes to the international preliminary examination report

a. ☐ is transmitted herewith.

b. ☐ is not required as the annexes are in the English language.

10. ☒ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115

a. ☐ was previously submitted by applicant on _____. *(Date)*

b. ☐ is submitted herewith, and such oath or declaration

i. ☐ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.

c. ☒ will follow.

II. Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. ☒ is transmitted herewith.

b. ☐ has been transmitted by the International Bureau.

Date of mailing (from form PCT/IB/308): _____.

c. ☐ is not required, as the application was searched by the United States International Searching Authority.

d. ☐ will be transmitted promptly upon request.

e. ☐ has been submitted by applicant on _____. *(Date)*

12. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:

a. ☒ is transmitted herewith.

Also transmitted herewith is/are:

☒ Form PTO-1449 (PTO/SB/08A and 08B).

☒ Copies of citations listed.

b. ☐ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).

c. ☐ was previously submitted by applicant on _____. *(Date)*

13. ☐ An assignment document is transmitted herewith for recording.

A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

_____

_____

_____

14. ☒ Additional documents:

a. ☐ Copy of request (PCT/RO/101)

b. ☒ International Publication No. WO 00/39560

i. ☒ Specification, claims and drawing

ii. ☐ Front page only

c. ☒ Preliminary amendment (37 C.F.R. § 1.121)

d. ☒ Other

PCT/IB/308, Preliminary Examination Report

15. ☒ The above checked items are being transmitted

a. ☒ before 30 months from any claimed priority date.

b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on _____, namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ Please charge, in the manner authorized above, the following additional fees that may be required by this paper and during the entire pendency of this application:

☒ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

☒ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☒ 37 C.F.R. § 1.17 (application processing fees)

☒ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☒ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

SIGNATURE OF PRACTITIONER

Reg. No.: 24,622

Clarence A. Green

Tel. No.: (203 .) 259-1800

(type or print name of practitioner)

PERMAN & GREEN, LLP

Customer No.: 2512

P.O. Address

425 Post Road, Fairfield, CT 06430 USA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Express Mail No : EL627430875US

In re Application of: SHINE et al.

INTERNATIONAL APPLICATION NO.: PCT/GB99/04438

INTERNATIONAL FILING DATE: 24 December 1999

U.S. SERIAL NUMBER.

TITLE. A METHOD OF TESTING A CELL SAMPLE

ATTORNEY DOCKET NO 768-010453-US(PAR)

Box PCT
The Commissioner of Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Dear Sir

Please amend the above-identified, patent application as follows:

IN THE SPECIFICATION.

After the Title and before the first paragraph, please insert the following new paragraph

This application claims the benefit of the earlier filed International Application No. PCT/GB99/04438, International Filing Date, 24 December 1999, which designated the United States of America, and which international application was published under PCT Article 21(2) in English as WO Publication No.WO 00/39560

IN THE CLAIMS

Please amend Claims 3, 4, 6, 8, 9, 13, 14, 15 and 16 as rewritten below.

3. A method according to claim 1, in which the property changed is that of the shape of the cells.

4. A method according to claim 1, in which the cell sample is subject to an alteration to cause the cells to sphere

6 A method according to claim 1, in which alterations in the cell population are detected by passing one or more aliquots of the cell sample through a sensor which is adapted to count the number of cells passing through the sensor.

8 A method according to claim 1, further comprising the step of pretreating the sample of cells to induce, or at least attempt to induce, agglutination

9 A method according to claim 1, in which the cell sample is obtained from a source of whole blood

13. A method according to claim 10, in which the antibodies from the different source are manufactured, or come from whole blood, plasma or serum.

14 A method according to claim 8, in which the sample of cells is pre-treated by exposure to heat

15. A method according to claim 8, in which the sample is warmed to a temperature of between 35^0C to 40^0C, preferably 37^0C

16 A method according to claim 8, in which the sample of cells is pre-treated by cooling the sample

REMARKS

In accordance with 37 C F R §1 121 (as amended on 11/7/2000) the rewritten claim(s) above are shown on separate page(s) marked up to show all the changes relative to the previous version of that section.

Respectfully submitted,

29 June 01

Clarence A Green Reg. No 24,622
PERMAN & GREEN, LLP
425 Post Road, Fairfield, CT 06430
(203) 259-1800
Customer No : 2512

Date

MARKED UP CLAIMS

3 A method according to claim 1 ~~or 2~~, in which the property changed is that of the shape of the cells.

4 A method according to any ~~preceding~~ claim 1, in which the cell sample is subject to an alteration to cause the cells to sphere.

6. A method according to ~~any preceding~~ claim 1, in which alterations in the cell population are detected by passing one or more aliquots of the cell sample through a sensor which is adapted to count the number of cells passing through the sensor.

8 A method according to ~~any preceding~~ claim 1, further comprising the step of pretreating the sample of cells to induce, or at least attempt to induce, agglutination

9. A method according to ~~any preceding~~ claim 1, in which the cell sample is obtained from a source of whole blood.

13 A method according to ~~any of~~ claims 10 ~~to 12~~, in which the antibodies from the different source are manufactured, or come from whole blood, plasma or serum

14 A method according to ~~any of~~ claims 8 ~~to 13~~, in which the sample of cells is pre-treated by exposure to heat

15. A method according to ~~any of~~ claims ~~8 to 14~~, in which the sample is warmed to a temperature of between 35^0C to 40^0C, preferably 37^0C.

17. A method according to ~~any of~~ claims 8 to ~~13~~, in which the sample of cells is pre-treated by cooling the sample

A METHOD OF TESTING A CELL SAMPLE

Background to the Invention

All types of blood cells occasionally agglutinate spontaneously, frequently heralding a serious haemolytic disease. It may indicate an underlying malignancy such as non-Hodgkin's lymphoma, Hodgkin's disease, acute lymphocytic leukaemia, carcinoma, thymoma and ovarian tumours. It occurs in blood group incompatibility as in haemolytic disease of the newborn, and mis-matched blood transfusions; also in paraoxysmal nocturnal haemoglobinuria and hypogammaglobulinemia; in some collagen diseases such as disseminated lupus erythematosis, rheumatoid arthritis, ulcerative colitis and hepatitis; in some infections such as viral and Mycoplasma pneumonia, cytomegalovirus, tuberculosis and infectious mononucleosis, and as a toxic reaction to some drugs such as L-dopa. As the presence of intra or extra vascular haemolysis in these diseases carries at least a 10% mortality, the identification of red cell agglutination is useful for the early diagnosis and for monitoring the response to treatment.

Traditionally, agglutination is detected by visually observing clumped cells. Whilst automated cell counters have supplanted all manual routine haematology they cannot detect agglutination sufficiently accurately to avoid manual verification. Indeed, existing automated cell counters erroneously measure agglutinated clumps of cells as one large cell producing an inaccurate mean cell volume and cell count and compound indices derived from them. An abnormally high mean corpuscular volume (MCV) or an abnormally elevated mean corpuscular haemoglobin concentration (MCHC) displayed by commercial haematology autoanalysers alerts the technician to the

possibility of the presence of agglutination. However, these indices are inadequate indicators of agglutination because they are not specific, moreover agglutination must rise to high levels before the indices exceed the normal limits. An elevated MCHC is produced by red cell fragmentation, lymphocytosis, hyperglycemia and haemoglobinaemia and therefore requires manual inspection and further testing to establish the diagnosis.

In conventional laboratories which perform blood typing and cross-matching, to determine the blood group of a sample one or two drops of existing commercially available blood group antibodies are added to neat whole blood, or more usually a 3 to 5% suspension of whole blood in normal saline. The suspension is incubated at room temperature for some minutes, typically 2 or 3 minutes. The suspension is then centrifuged for 30 to 45 seconds at 3000 rpm in a bench-top centrifuge. The suspension is then gently shaken for a few seconds. The tube is then examined visually for the presence of agglutinated cells and confirmed using low-powered microscopy. Cross-matching is performed in the same way using the recipient's plasma as the antibody in place of commercial antibody to confirm that there is no agglutination.

Summary of the Invention

According to the present invention, a method of detecting agglutination in a sample of cells comprises the steps of inducing cells to change at least one of their properties so as to separate agglutinated cells and detecting the resultant alteration in the cell population.

Preferably, the property change is that of the shape of the cells. More preferably, the cell sample is subject to an alteration in environment to cause the cells to sphere. In a preferred example, the alteration in the environment is a change in osmolality of a liquid medium in which the cells are suspended, preferably by the addition of water.

Preferably, alterations in the cell population are detected by passing one or more aliquots of the cell sample through a sensor which is adapted to count the number of cells passing through the sensor. More preferably, the sample is fed continuously into a solution the osmolality of which is changed continuously to produce a continuous series of aliquots of cells which are passed through the sensor.

Preferably, the method further comprises the step of pre-treating the sample of cells to induce, or at least attempt to induce, agglutination. In one preferred use of the invention, a cell sample of unknown antigenicity from one source is mixed with antibodies from a different source. The antibodies may be manufactured or come from whole blood, plasma or typically serum. In a further step, the putative antigen-antibody mixture may be tested at different temperatures to reveal heat sensitive agglutination. Accordingly, a new test is provided which can replace existing blood grouping and cross matching techniques.

The present invention measures agglutination using a process which is also capable of testing how tightly agglutinated cells are bonded by measuring how much force is required to separate them. This property depends upon antibodies interacting with the complement system. Agglutination of red blood cells is a function of the type and

number of antigen combining sites on the surface of the cells, which bind with complementary Gig antibody molecules. The strength of agglutination is a function of the proximity of the binding sites on the cell surface. By placing a whole blood sample into a typically 1:10,000 suspension, and causing cells which are approximately bi-concave discs to sphere, the effective surface area available for bonding diminishes. Sphering a cell increases the space between antigen binding sites and increases the mean distance across which bonding occurs. The surface area available for bonding between cells decreases as cells sphere hence they lose bonding strength and separate. By recording the inducing pressure and the number of cells (or quantities related to it) as they change with respect to the inducing pressure, agglutination can be detected, quantified and monitored. Cells which have agglutinated, when tested by this method, separate and thereby increase the cell count in a characteristic fashion. In a further step the sample is subject to mechanical agitation which tends to promote agglutination in normally shaped cells capable of agglutination but promotes separation of spherically shaped cells.

Brief Description of the Drawings

Examples of the present invention will now be described in detail with reference to the accompanying drawings, in which:

Figure 1 is a screen dump of a set of results from an automatic blood cell analyzer of the type described in detail in International patent application WO97/24601, for a patient having normal non-agglutinated blood cells;

Figure 2 is a similar screen dump of a set of results for a patient having agglutinated blood cells; and,

Figure 3 is another screen dump showing the results of mixing a sample of blood with antibodies in a test to determine blood type.

Detailed Description

The method of the present invention is exceptionally useful in conjunction with the methods and apparatus described in the applicants' earlier filed International patent applications, namely WO97/24601, WO97/24598 and WO97/24599, and enhances the general utility of the tests described therein.

The preferred method consists of counting the cells as they pass through an aperture. The instrument may be configured with a mixing chamber into which saline, cells and diluent are injected, in which case the number of cells passing through the aperture at every osmolality does not vary. When only two streams are injected into the mixing chamber, diluent and a saline suspension into which the cells have been previously introduced, the number of cells passing through the aperture is fixed at a level that is directly proportional to the osmotic gradient. Since the red blood cells suspended in a liquid medium are exposed to a progressive reduction in ambient osmolality, and the method normally injects a progressively smaller stream of cells into the mixing chamber, a progressive reduction in cell count is observed.

The results generated by the instrument described in International Patent Application WO 97/24601 for a normal patient are shown in Figure 1. In Figure 1, as well as in Figures 2 and 3, in area A the plot represents the red cell count. Deviation from the predetermined straight line of cell count against osmolality (as shown in area A of

Figure 2) can only occur if additional particles appear, or are stimulated by the ambient change in pressure.

As will be described below, when cells agglutinate or are made to agglutinate, the cell count falls; then, as the cells sphere, the cell count increases with each aggregate tending to separate into its component parts in inverse proportion to the strength of the agglutination.

Most cells sphere in the range of pressures in the interval between P_{max} and P_0, where P_{max} is the point at which the rate of fluid flow into the cell reaches a maximum and P_0 is the equilibrium point (see area B). If agglutinated clumps are present they will separate in the same interval causing a local increase in count. Furthermore, the point at which P_0 occurs gives an indication of whether or not agglutination is occurring, since the point at which P_0 occurs increases if cells are agglutinating.

Our corresponding International application (Agent's reference G14201WO) discloses a method of measuring cell fragments. Fragments and disrupted agglutinated cells (DACs) can be segregated by size. Fragments are quite small between 10 and 30 fl in volume whereas DACs are at least three times the size, generally between 60-110 fl. In addition, the isotonic MCV is normal or reduced in the presence of fragments while the MCV is elevated with agglutination. As the normal range of MCV is so large it can hide much agglutination.

Sample ageing and the application of mechanical, ultrasound or other stress increases

the count of intact cells if the sample was agglutinating and decreases the number of intact cells if the sample is fragmenting. Dropping the ambient osmolality below P_0 has no further disrupting effect on agglutinated clumps but the frequency of cell fragments have been found to vary inversely with osmolality.

Figure 2 shows the results for a patient having agglutinated blood cells. The sudden increase in cell count at sphering is shown clearly in area A, and the increased sphericity index (SI) appears as a fat cell in area B. SI can also be seen from the Table (area C). A sphere has a SI of 10 whereas a flatter cell has a higher SI. In Figure 2 (abnormal patient) the value of SI is 10.24 whereas in Figure 1 (healthy patient) the corresponding value is 14.37.

Area D in Figure 2, in comparison with Figure 1, shows the increase in variance of the red cell frequency distribution due to agglutinated clumps of cells. An analysis of the frequency distribution provides an indication of whether or not the cells are agglutinating. Firstly, the width of the distribution, as measured by the standard deviation (SD), or coefficient of variation (cv), increases with agglutination. Secondly, any deviation from a normal distribution can be measured. A bias away from the centre leading to a flatter shaped curve, termed negative kurtosis, provides an indication of agglutination. Comparing area D in Figures 1 and 2 shows that in the abnormal patient the standard deviations are about twice the normal and kurtosis is negative.

Area E shows frequency distributions indicating the profile of cell size measurement

against increasing osmolality. As the stress is increased the cells begin to swell resulting in the gradual increase in mean cell size. At P_0 the cell size can increase no more, and upon a further increase in stress, the cells evacuate their contents to become "ghost cells".

As an example, the method may be embodied into an instrument for the automated recognition of blood groups and cell types by the induction and detection of agglutination by introducing antibodies (for example, using any one or more of the commercially available antibodies currently used for blood typing purposes, or using the recipient's plasma as the antibody source for the purpose of cross-matching) into the water syringe which subsequently meets the saline blood suspension in the mixing chamber. Agglutination caused by the interaction of the antibodies and the antigens on the surface of the cells, or the lack of it, is detected at the sensor aperture by counting. This test eliminates the need for manual blood grouping and cross-matching.

The results generated by such an instrument described in International patent application WO97/24601 are shown in Figure 3. In this example, the fluids were warmed within the apparatus to a temperature of around 37°C ie body temperature, to stimulate normal body environment. Agglutination is recognisable by the presence of an increase in the red cell count, usually between P_{max} and P_{min}, by the frequency distribution (in this example the isotonic, spherical, ghost, and "user" selected frequency distributions are taken at respective sampling instants shown by the "H"s in area E) showing negative kurtosis, by an increase in the distribution width of the

cell population measured by an increase in the standard deviation or coefficient of variation, by an increase in the sphericity index, and by an increase in the osmolality which induces zero permeability (P_0). Any change, even minor change (detectable when compared with a control run without antibodies) must be attributable solely to the antibody. In this particular example, a second population of cells is visible on both areas B and E in Figure 3, which also indicates agglutination.

The present invention is particularly useful in the early detection of agglutination, hence the early detection and subsequent treatment of haemolytic diseases, and enhanced possibility of recognizing the underlying pathology. It is also possible to quantify the strength of cell agglutination from the extent to which separation is achieved and the ease with which it is achieved. As the unagglutinated cell concentration is known any reduction in the isotonic count represents agglutination. As the cell suspension is exposed to the sphering gradient, the original count will be restored at higher osmolalities and in proportion to the strength of the agglutination. Finally, the method provides for the automatic identification of blood groups and cell types by inducing cells to agglutinate and subsequently testing them using the method.

CLAIMS

1. A method of detecting agglutination in a sample of cells, comprising the steps of inducing the cells to change at least one of their properties so as to separate agglutinated cells and detecting the resultant alteration in the cell population.

2. A method according to claim 1, comprising the step of measuring the force required to separate agglutinated cells.

3. A method according to claim 1 or 2, in which the property changed is that of the shape of the cells.

4. A method according to any preceding claim, in which the cell sample is subject to an alteration to cause the cells to sphere.

5. A method according to claim 4, in which the alteration is a change in osmolality of a liquid medium in which the cells are suspended.

6. A method according to any preceding claim, in which alterations in the cell population are detected by passing one or more aliquots of the cell sample through a sensor which is adapted to count the number of cells passing through the sensor.

7. A method according to claim 6, in which the sample is fed continuously into a solution the osmolality of which is changed continuously to produce a continuous

series of aliquots of cells which are passed through the sensor.

8. A method according to any preceding claim, further comprising the step of pretreating the sample of cells to induce, or at least attempt to induce, agglutination.

9. A method according to any preceding claim, in which the cell sample is obtained from a source of whole blood.

10. A method according to claim 9, in which the sample of cells are treated with antibodies from a different source.

11. A method according to claim 10, in which the cells are treated in order to determine the blood type.

12. A method according to claim 10, in which the cells are treated in order to cross-match the sample.

13. A method according to any of claims 10 to 12, in which the antibodies from the different source are manufactured, or come from whole blood, plasma or serum.

14. A method according to any of claims 8 to 13, in which the sample of cells is pre-treated by exposure to heat.

15. A method according to any of claims 8 to 14, in which the sample is warmed to a temperature of between 35°C to 40°C, preferably 37°C.

16. A method according to any of claims 8 to 13, in which the sample of cells is pre-treated by cooling the sample.

WO 00/39560 PCT/GB99/04438

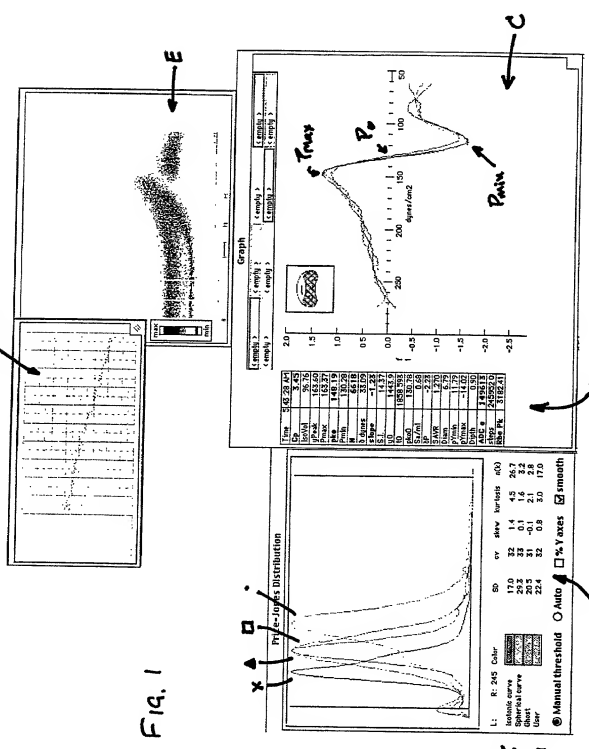

FIG. 1

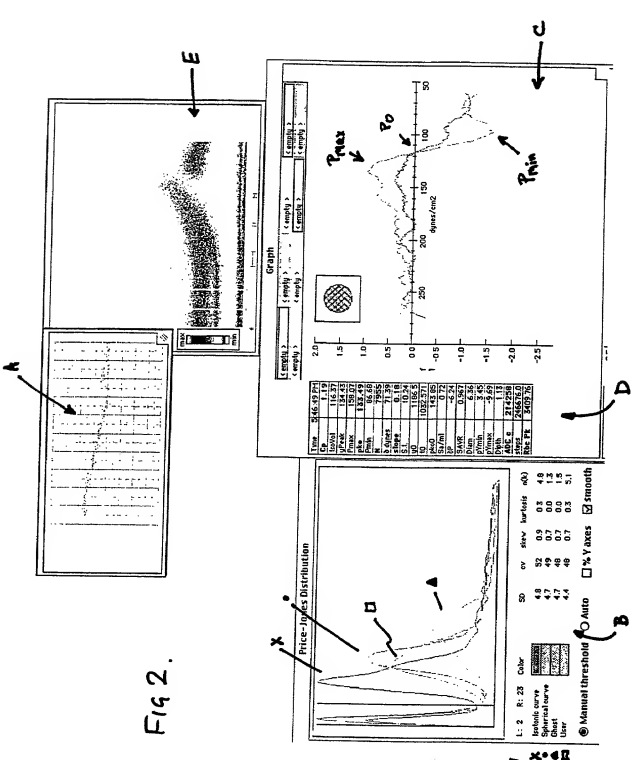
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Fig 2.

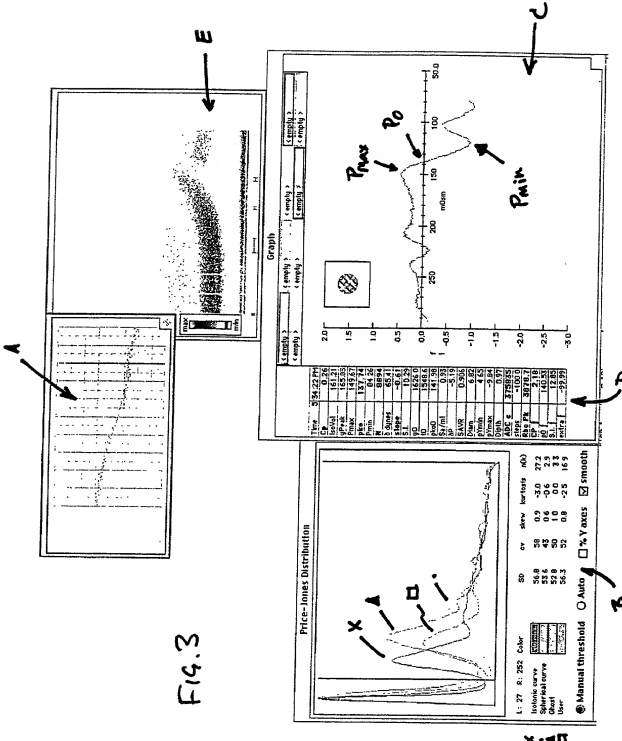
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FIG. 3

Docket No. 768-010-53-US(PAR)

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

English Language Declaration

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Title: A METHOD OF TESTING A CELL SAMPLE

the specification of which

(check one)

☐ is attached hereto

☒ was filed on as United States Application No 09/869,597 or PCT International Application Number PCT/GB99/04438 filed on 24 December 1999

and was amended on (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)	Priority Not Claimed
PCT/GB99/04438	PCT	24 December 1999	☐
9828765.9	United Kingdom	29 December 1998	☐
			☐
			☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below

(Application Serial No.) (Filing Date)

(Application Serial No.) (Filing Date)

(Application Serial No (Filing Date)

I hereby claim the benefit under 35 U.S.C Section 120 of any United States application(s), or Section 365(c) of any PCT International Application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International Application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C.F.R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name and registration number)

All attorneys listed under Customer No. 2512

Send Correspondence to:

Customer No.: 2512

Direct Telephone Calls to: (name and telephone number)
Clarence A. Green, Reg. No. 24,622 (203) 259-1800

Full name of sole or first inventor:
Ian Basil SHINE

Sole or first inventor's signature: Ian Shine DATE: 18 July 01

Residence:
444 Central Park West, New York, New York 10025 USA

Citizenship:
British NY

Post Office Address:
444 Central Park West New York New York 10025 USA

Full name of second inventor:
Thomas Adam SHINE

Second inventor's signature: Tom Shine DATE: 18 July 2001

Residence address:
3 Crawford Street, No. 5, Cambridge, Massachusetts 02139 USA

Citizenship:
British MA

Post Office Address:
3 Crawford Street, No. 5, Cambridge, Massachusetts 02139 USA